

ON GOLD CHLORIDE AS A STAINING AGENT FOR NERVE TISSUES.¹

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THE object of the present paper is a consideration of the chemical relations of gold chloride, as it is used in staining nerve fibres and ganglion cells. This is in effect a continuation of two articles on the same subject, already published in the JOURNAL OF NERVOUS AND MENTAL DISEASE; in order to give as complete a view of the subject as is possible, a partial repetition will be necessary.

The Freud method, which has been in use for some years, is as follows: The section, cut from tissue hardened in Müller's fluid, comes first into a 1% solution of gold chloride, where it remains for two hours. It is then brought successively into a 20% solution of potassium hydrate, and a 10% solution of potassium iodide; in the latter solution in a few moments it assumes a reddish tint. If successful the nerve fibres and ganglion cells are sharply stained. Or occasionally, for some unknown reason, the myeline sheaths alone are stained, presenting an appearance much like that of a section stained according to the Weigert method. This stain has been little used, as it is said to be quite unreliable.

Now, the formation of the red or purple oxide of gold from the chloride is a chemical process, and as such follows fixed laws. It is the result of the combined or consecutive action of oxidizing and reducing substances on the chloride; so that it is probable *a priori* that if we are sufficiently well acquainted with the substances used in the reaction, and the way in which they act, so that we can

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make the conditions in all cases practically identical, we shall be able to obtain uniform results.

The objects of the investigations summed up in the present paper are two-fold ; namely, to find chemical agents which shall determine a formation of gold oxide in the tissues in such a way as sharply to differentiate them ; second, to determine the mode of action of these agents, so as to make the methods of their application reliable.

The method which, in a previous article, I have designated as No. 1, is briefly as follows :

METHOD NO. 1.

The section cut from tissue hardened in Müller's fluid (or much better in a simple 2% solution of potassium bichromate) is brought for a time varying from ten minutes to several hours, into a 1% solution of gold chloride ; then for a moment into water, and into a 10% solution of potassium hydrate, where it remains for 1-2 minute ; then, after a moment in water, into a reducing fluid, consisting of

Sulphurous acid, 5 cc.
Tr. iodi 3%, gtt. x.
Liq. ferri. chlor., gtt. i-iii.

This method depends for its success, in the first place on the presence of a chrome salt in the tissues. Hardening in Müller's fluid is essentially a reduction of the potassium bichromate by the fresh tissue, with an appropriation by the tissue of chromium, probably in the form of an oxide.

That the chrome salt is deposited in such a way as to differentiate the nerve elements, is seen by a glance at the cut surface of a bit of nerve tissue hardened in potassium bichromate. The tracts of nerve fibres which show on the surface may be followed with great accuracy ; to make this differentiation available for microscopical purposes, it is only necessary to replace the chromium by another metal, whose oxide has a sufficiently intense color to be plainly visible when under the microscope.

It is this oxide of chromium, which is replaced by gold chloride when the section is brought into the gold solution,

and if it has been soaked out of the tissue in alcohol or water, no stain can be obtained. This necessitates a staining of the section within a reasonable time after the removal of the tissues from the bichromate solution.

In the second place, the stain is dependent on the presence of extraneous matters in the gold solution. Pure gold chloride, dissolved in distilled water and carefully neutralized, stains little if at all. It is not too much to say, that every section which has been successfully stained by the older methods, has been so by virtue of hydrochloric acid or some other impurity accidentally present in the gold solution. The commercial chloride of gold almost always contains hydrochloric acid, often also chloride of copper, and a solution which is in use soon contains varying quantities of chrome salts, acquired from the sections which have been brought into it.

Much better results may be obtained by adding substances designedly to the gold solution, and these substances should be, in general, either acids or metallic salts, or both.

The acid which is most useful alone is hydrochloric acid ; those which may be used with the metallic salts are hydrochloric, nitric, sulphuric and chromic acids.

The metallic salts which are most useful are ammonium vanadate, potassium permanganate, sodium stannate, and others.

In this method, No. I, the potassium hydrate may be used in solution alone, but if it is so used the stain is often too diffuse. In this case substances may be added to differentiate the color, giving a sharper stain to the nerve elements proper, and leaving the glia tissue little stained, if at all. Ferricyanide of potassium is the most powerful agent of this class. The ferrocyanide acts in the same way, but more weakly.

There is another principle which it is necessary to bear in mind in all operations with metallic salts ; it is this : chemical agents which have just been set free are very active, and ready to enter into new combinations. In order that they should be vigorous in their action, the reducing

fluid should be freshly made for each set of sections to be stained, and the potassium ferricyanide should have been recently added to the hydrate solution. The gold solution, on the other hand, may be used for an indefinitely long time.

It is evident that with all of these means at command for varying the process, the number of different combinations which may be employed is endless. It will suffice to give one of them in detail.

The section to be stained is brought from water into a 1% solution of gold chloride, to which has been added 2 or 3% of hydrochloric acid. Here it remains for two hours, more or less; after being washed superficially in water it is then brought for $\frac{1}{2}$ to a minute into 10 cc. of a 10% solution of potassium hydrate, to which has just been added a piece of ferricyanide of potassium as large as a pin-head; it is better to add the latter salt in the solid form, and to ensure rapidity of solution it may be kept powdered and a quantity about corresponding to the above dissolved in the potash solution.

The section after this may be brought for a few moments into a 10% sol. of pot. hydrate to remove the excess of ferricyanide, and then, after washing a moment in water, comes into the reducing fluid made as follows:

Sulphurous acid, 5 cc.
Tr. iodi, 3%, gtt. x.
Liq. ferri. chloridi, gtt. i.

The section at once assumes a bright, purplish red color, and in a moment or two should be removed to water, and mounted in balsam by the usual manipulations.

The quality of the stain as affected by variations in the process will be considered later.

The above may be taken as a type of the process in Method No. 1.

In order to have well in hand the manipulations which may advantageously be used, it is well to begin with a series of sections cut from a bit of well-hardened spinal cord, and, after staining as above, to try the effect of adding dif-

ferent acids and metals alone and in combination, to different portions of the gold solution, and testing the staining powers of each. A considerable variety of results will be obtained in this way, and the methods thus learned may be adapted at will to the state and quantity of chrome salt in any given set of sections.

METHOD NO. 2.

This differs from Method No. 1 mainly in the fact that the reducing fluid contains a salt of tin. This change, however, necessitates a few variations in the other steps of the process.

To the gold solution may be added a metallic salt, or a salt and an acid.

To the hydrate solution should be added, in place of the ferricyanide, a trace of ammonium vanadate, and potassium permanganate; the latter substance has the same differentiating power that is possessed by the ferricyanide in the preceding method.

On the whole this method gives better results than does the preceding one; it should be varied to suit differences in the hardening, since it is obvious that a section thoroughly impregnated with chrome salt from a long stay in the bichromate solution, may endure, and indeed require a more vigorous differentiating or decolorizing action than would a tissue not so well hardened.

The method adapted to a moderately hardened tissue is as follows:

To 5 cc. of a 1% solution of chloride of gold, add one or two drops of a 1% solution of potassium permanganate, and one drop of nitric acid; this solution is permanent, and at any rate should not be used until two days after it is made.

The section, after two hours in this gold solution, is brought for a moment into water, and then into 10 cc. of a 10% potassium hydrate solution, to which has just been added a trace of ammonium vanadate and one or two drops of a 1% solution of potassium permanganate. Here it re-

mains for $\frac{1}{2}$ to a minute, is brought for a moment into water, and then into the reducing fluid, made as follows :

Tin solution,	gtt. xv.
Water,	3 cc.
Iron solution,	gtt. ii.
Sulphurous acid,	3 cc.

What has been called tin solution above, is made by adding 3% tincture of iodide to a small amount of pure protochloride of tin, until in the solution the reddish color of the iodine begins to be apparent.

The iron solution is a saturated aqueous solution of phosphate of iron, the soluble scale salt being employed.

The section almost at once assumes a deep purple color in the reducing fluid, and is removed to water and mounted in balsam as before.

When the sulphurous acid is added to the reducing fluid, an active chemical change takes place, and a heavy precipitate falls ; it is while this reaction is going on that the solution is most efficacious, and the section should be brought into it at that time.

Sulphurous acid is a gas, which occurs in commerce dissolved in water ; it must be kept in tightly glass-stoppered bottles, and should be obtained as fresh as possible.

With tissues which have been long and thoroughly hardened, a gold solution may be employed containing, instead of the above, ten drops of a saturated solution of ammonium vanadate, and two drops of hydrochloric acid, to 5 cc. of a 1% gold chloride solution.

QUALITY OF THE STAIN.

This varies considerably, according to the care with which the various steps of the process, and especially the hardening, are carried out. In the comparatively unimportant matter of the exact shade of color taken by the nerve elements, it is not always possible to predict the result, as a slightly greater or less intensity of reducing action may change the result from reddish to bluish purple, and *vice versa*. In general it may be said that hydrochloric acid in

the gold solution gives a reddish color in the section, and that metals without acids in the gold solution give a more bluish shade.

Without doubt gross errors in the staining process proper are capable of preventing a useful result from being obtained, but if with moderate care a good result cannot be obtained in test sections by either method, the trouble is invariably in the hardening of the tissue.

In case of such failure to obtain good results, the possibility should first be considered, of the chrome salt having been soaked out from the tissues, as in that case the task of obtaining a good stain is quite a hopeless one. Next the hydrate solution should receive attention, as too large a quantity of ferricyanide or other substance added to it is quite capable of decolorizing not only glia, but nerve tissue as well. Attention to these two points will, with a moderately well hardened tissue, be sufficient to insure success.

As with all other methods, the best results are only obtained by careful handling of thoroughly well hardened tissues.

HARDENING.

The object of the hardening process should be clearly borne in mind.

The Weigert method depends for its success on the presence of a chrome salt acting as a mordant in the myeline sheaths ; tissues hardened for a long time in Müller's fluid often appear dark green, almost black, in the white matter, a light gray color in the gray matter ; these tissues stain well by the Weigert method ; they give with gold chloride a distinct myeline sheath, but weak ganglion cell and axis cylinder stain.

The object to be attained in hardening tissues for the Golgi stain is a comparatively rapid permeation of the lymph spaces ; the appropriation of the chrome salt by the tissue itself appears to be not so important.

Quite fresh tissues hardened for a long time in a weak solution of bichromate of potash, present no very marked difference in color between the white and gray matter ; but in the gray matter may be seen, on the cut surface of a bit

of spinal cord, the groups of ganglion cells, and bundles of nerve fibres radiating out into the white matter. Tissues hardened in this way give with gold chloride a clear ganglion cell and axis cylinder stain, but probably stain badly by the Weigert method. They stain well with carmine and nigrosine.

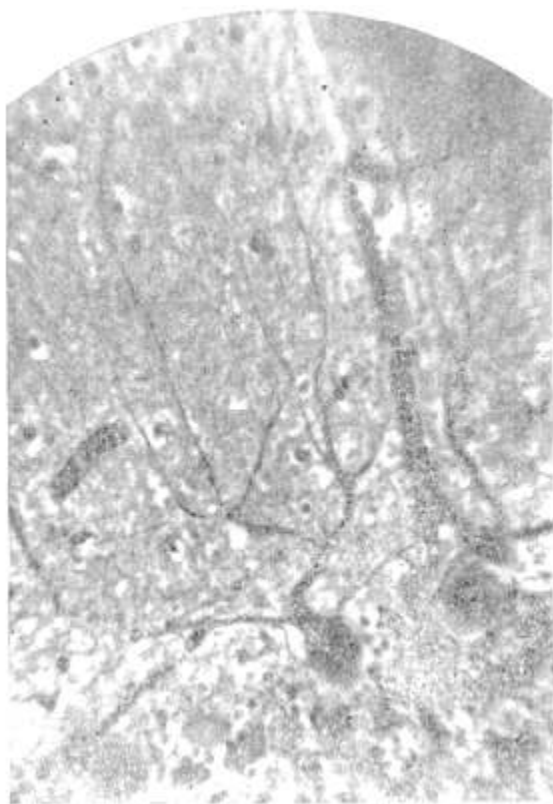


FIG. 2

Fresh tissues should be brought for a week into a 1%, then into a 2% solution of potassium bichromate, and should remain in the latter for from three to six months or longer; the hardening in this way proceeds more slowly than when sodium sulphate is present, but the chrome salts penetrate much better, and act more uniformly throughout the tissues.

The tissues should be cut into rather small pieces, and the solution changed often.

That only well-hardened tissues can be successfully stained with chloride of gold is by no means so disadvantageous as at first sight appears. Even gross tissue changes are often obscured, and what is worse simulated, by *post-mortem* change both before and after the commencement of the hardening process, and this no matter what stain is

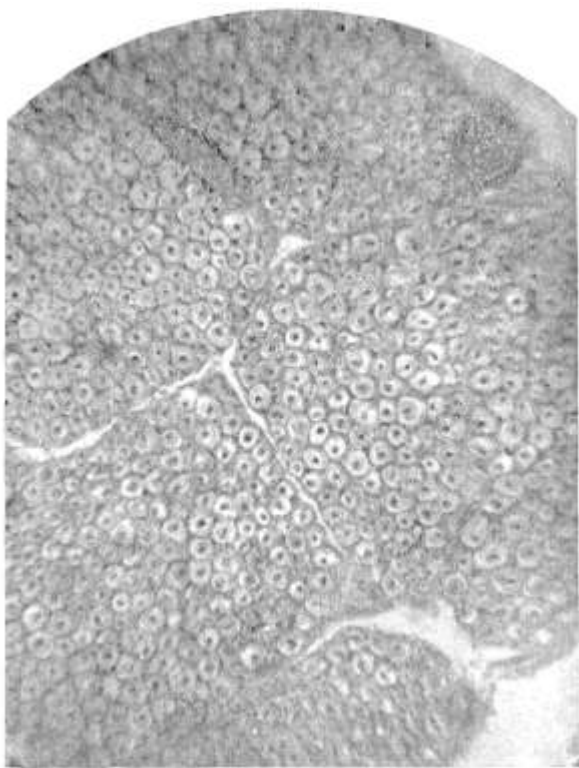


FIG. 1

used ; the finer nerve fibres are not very unlike the surrounding glia in chemical constitution ; it is probable that many forms of disease are due to a finely molecular or chemical change in the relations of these parts, and any attempt to investigate such conditions must be undertaken in tissues which have been preserved as nearly as possible in the relations which obtained during life.

The merit of gold chloride is not that it is easily availa-

ble in tissue no matter how badly hardened, but that with care nerve elements may be more sharply differentiated than with carmine or nigrosine, and I believe with as uniformly good results.

The accompanying photo-micrographs are from untouched negatives taken from sections stained by the above methods. They were taken with a Zeiss microscope, objective DD, eye-piece No. 3, and a Schmidt detective camera



FIG. 3

without lens. The plate was about six inches from the eyepiece. An oil lamp furnished the light, which was passed through an Abbé illuminating apparatus. The plate used was a Seed 26 extra rapid, and the exposure two to five minutes. The half-tone reproductions are not so clear as is a bromide contact or albumen print.

The first micrograph is from a section of the normal human cerebellum, and shows several cells of Purkinje, with

their processes extending into the gray matter. The second is a cross section of a human nerve trunk, taken from the spinal canal. The third is from the spinal cord of an ox, and shows a large motor ganglion cell in the anterior horn of gray matter, surrounded by other cells and nerve fibres. The tissues were all normal, hardened in Muller's fluid, and stained, No. 2 by the first method, the others by the second method.

SOME SEDATIVES FOR INSANE PATIENTS.

Dr. O. Dornblüth (Kreuzberg), *Therapeut. Monatshefte*, 1889, Aug. —; *Neurolog. Centralblatt*, No. 4, 1890). Dornblüth experimented with hyoscine hydrobromate (E. Schering), and obtained the same results as with Merck's preparation. The remedy was mostly given internally in soup, coffee and wine, in doses of 2 neg. two to three times daily. As a secondary action vertigo, a tired feeling and dryness of the throat were observed; illusions were only seen once and in a nervous woman who mentally was normal. After subcutaneous employment of the drug quite frequently a loss of control of the extremities, dryness of the throat, etc., appeared. The action of subcutaneous injections, as a rule, is observed after three to ten minutes, while that after the remedy is internally administered in one-half to one hour, if the patient be kept quiet after taking the drug. In ten per cent. of the cases an initial stage of excitement, sometimes of two to three hours, would precede. The remedy was especially valuable in periodic mania and continuous maniacal states; also in epileptics, hallucinatory patients, paralytics, and patients suffering from dementia, it acted sedatively. He also experimented with codeinum purum and phosphoricum, subcutaneously, in doses of 0.025 to 0.05; internally, 0.02 to 0.08. In quiet melancholic patients, doses of 0.06 to 0.08, two to three times daily, acted still better than the "course" of opium already tried. In maniacal conditions of excitement codein acted not so well as hyoscine. It acted very well in anxious restlessness, præcordial sensations and sleeplessness. The patient, according to Fischer-Kreuzlingen, does not form a codeine habit. The phosphate is only to be used subcutaneously, as codeinum purum is soluble with difficulty. No secondary or disagreeable after-action appeared. In a slight degree of excitement he began with 0.02, and with grave excitement 0.04.